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Production of Algae Containing 93% Carbon-13

by

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CONTAINING 93% CARBON-13

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ABSTRACT

Carbon-13 has been biologically incorporated into *Chlorella pyrenoidosa* to the 93% level with no observable induction period or gross changes in cell morphology or size.

The cultivation and usefulness of isotopically altered organisms and compounds extracted from them have been reported by Katz, et al.[1,2,3,4] Algae have been cultured in D_2O and H_2O systems using low levels of $^{13}CO_2$ in conjunction with ^{15}N and ^{18}O[5] for purposes of electron paramagnetic resonance[6] and nuclear magnetic resonance[4] studies.

This paper reports the first growth of algae (*Chlorella pyrenoidosa* - Sorokin strain) on a mineral salts medium[7,8] using 94.7 atom % $^{13}CO_2$[9] as the sole source of carbon.

C. pyrenoidosa has been successfully cultured through 15 generations (three consecutive runs of 5 generations) in a 5 liter oscillating culture apparatus. This experiment was repeated; results were comparable to those of the first experiment.[10] The organism was further grown in mass cultures of 25 liters through 16 generations.[11]

The ^{13}C content of the algae, determined by mass spectrometry, reached 90% with the first five generations of growth. Growth rate data (Fig. 1) and serial ^{13}C incorporation determinations (Fig. 2) through the first five generations show no induction period or growth rate differences when compared with *C. pyrenoidosa* which were cultured using CO_2 having natural isotopic abundance of carbon.

After the first five generations of growth on ^{13}C, however, the rate of growth changed. Least square analyses of the hyperbolic growth curves demonstrate that the slopes of the curves for growth on natural abundance CO_2 ($m = 0.2528$) and for growth through the first five generations ($m = 0.2582$) on 94.7 atom % $^{13}CO_2$ are statistically the same. Comparison of the growth rates for the previously referenced curves with growth on 94.7 atom % $^{13}CO_2$ beyond the first five generations of growth on ^{13}C shows a dramatic 15 to 20% growth rate increase. The slopes of the curves were 0.2582 for the first five

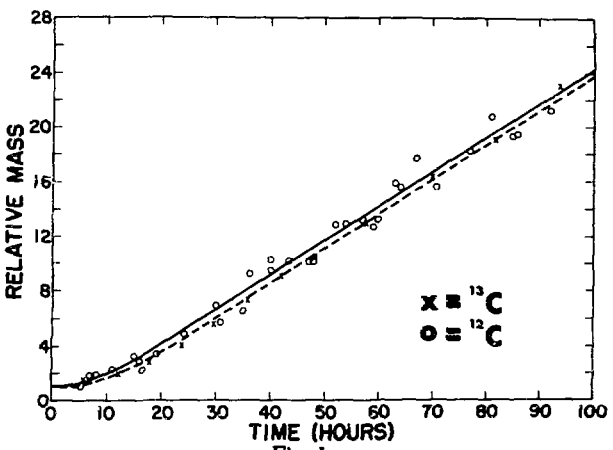

Fig. 1.

Least squares fit (Ref. 13) for relative masses of C. pyrenoidosa produced on natural abundance CO_2 and 94.7 atom % $^{13}CO_2$ with time. Shown are the scattering of empirical points along the fitted curves for growth rate using natural abundance CO_2 (0) and growth on 94.7 atom % $^{13}CO_2$ (X) through the first five generations. The slope of the hyperbolic growth curve (asymptote) for ^{12}C growth is 0.2528 and that for growth on ^{13}C through the first 5 generations is 0.2582. The slopes are the same at the 95% level of confidence.

generations and 0.2936 after the first five generations. The difference is significant at the 95% level. This apparent stimulation is being investigated at this time.

The growth stimulatory effect observed at the 90 to 93% level of ^{13}C incorporation may continue as the percentage of incorporated ^{13}C is increased; however, it will be necessary to bring the level of incorporation to the 99+% level before final conclusions can be drawn.

R. A. Uphaus[8] has indicated that the cytological and morphological abnormalities caused by the incorporation of 99.7% ^{2}H into *C. vulgaris* might be partially alleviated by high concentrations of ^{13}C; it is possible that his observations are explained by the above reported growth stimulation of incorporated ^{13}C at the 90+% level.

The algae continue to grow normally after 290 hours (15 generations) of continuous culture at the 93% ^{13}C level. Culture densities of 5 grams per liter (lypholized) have been obtained. Photomicrographs[12] taken at six-hour intervals during growth indicate no gross morphological changes; cell sizes average about 3 μm as shown in Fig. 3. This observation in which ^{13}C is the sole isotope substituted differs from those of Katz, et al[5] where gross changes in morphology and cell size distribution were reported in algae grown in D_2O systems having various isotopic compositions of carbon, oxygen and nitrogen.

Organisms labeled at high levels with ^{13}C can serve as a valuable source of metabolites for use in such areas as elucidation of the structure of biopolymers, kinetics of enzyme reaction, biosynthetic isotope effects, rate of radiation damage and reconstitution of biologically important systems, and in human clinical applications where the radiation from ^{14}C may be undesirable.

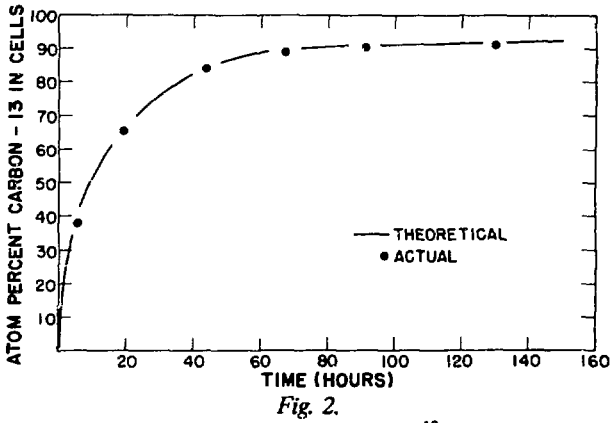

Fig. 2.

Theoretical versus Actual Rate of ^{13}C Incorporation. The curve shown is the theoretical rate of ^{13}C incorporation based upon the theory that each generation of cells contains one-half the parent cell carbon plus that quantity which would be incorporated assuming no ^{13}C isotope effect. Also shown are the plotted points which are the actual ^{13}C incorporation data obtained by mass spectrometry. These data show that through the first five generations there is no difference, within experimental error, between the actual and theoretical rates of ^{13}C incorporation.

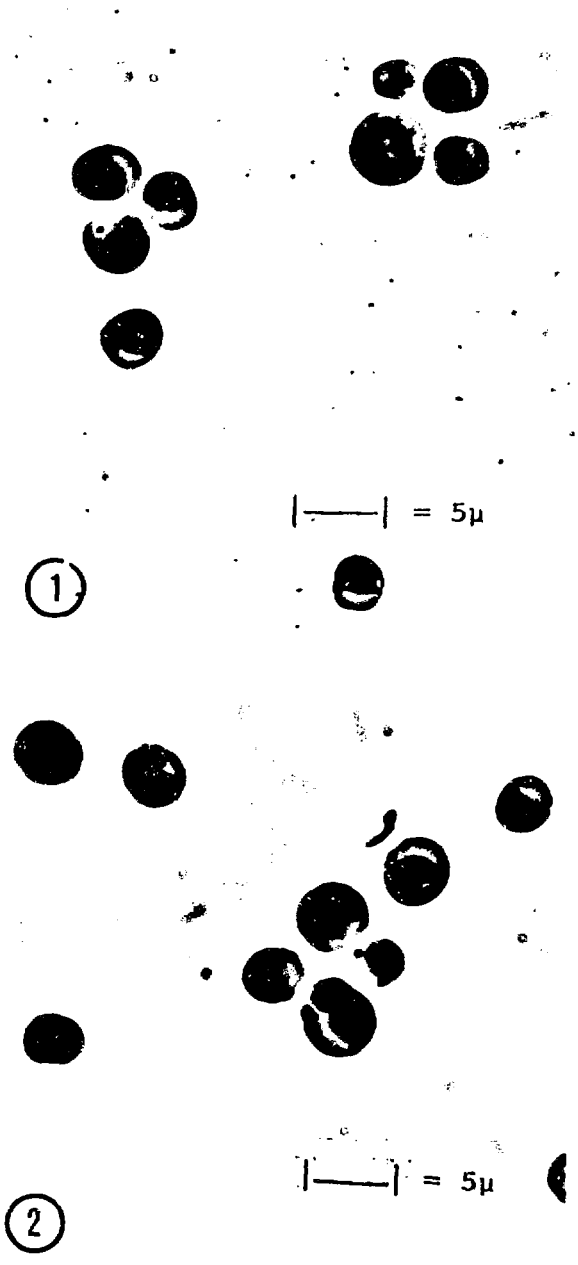

Fig. 3.

Photomicrographs showing gross morphology and distribution of cell size. 1, C. pyrenoidosa grown using normal CO_2; 2, C. pyrenoidosa grown using 94.7 atom % $^{13}CO_2$: photograph taken at the end of 290 hours culture time. Algal cells were suspended in the growth medium for photographing.

References

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3. Homer F. Daboll, *et al.*, Biotechnology and Bioengineering IV, 281 (1962).

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6. D. H. Kohl, *et al.*, Nature 206, 1105 (1965).

7. Composition of algal culture medium was that used by C. Freeman Allen, Department of Chemistry, Pamona College, Claremont, CAlifornia and modified to fit the growth requirements of *C. pyrenoidosa*.

8. Recent private communications with R. A. Uphaus at Argonne National Laboratory indicate that he and his associates have successfully cultured *C. vulgaris* on ^{13}C to about the 90% level of incorporation.

9. Dale E. Armstrong, *et al.*, Los Alamos Scientific Laboratory of the University of California, LA-4391. We thank Eugene S. Robinson, B. B. McInteer and Robert M. Patten for supplying the $^{13}CO_2$.

10. V. H. Kollman, C. W. Christenson and E. B. Fowler, in preparation.

11. J. R. Buchholz, V. H. Kollman, C. W. Christenson and E. B. Fowler, in preparation.

12. Photomicrographs were prepared by Juliamarie Langham, Los Alamos Scientific Laboratory.

13. Least square analyses were completed by Gary L. Tietjen, T-Division, Los Alamos Scientific Laboratory.